

Please insert the accompanying paper copy of the Sequence Listing, page numbers 1 to 3, at the end of the application.

REMARKS

In accordance with 37 C.F.R. §§1.821 to 1.825, Applicants request entry of this amendment. This amendment is accompanied by a floppy disk containing SEQ ID NOS:1-11, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.

Attached hereto is a marked-up version of the changes made to the Specification by the current Amendment. The attached pages are captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE.**"

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**In the Specification:**

Paragraph beginning at line 14 of page 7 has been amended as follows:

Figure 2 provides a schematic representation of the differentially methylated region (DMR) of the human *IGF2-H19* region. The two 450-bp repeat (A1 and A2) and seven 400-bp repeat (B1–B7) units are shown. The potential methylation sites on the upper strand DNA of the studied region are represented by open circles. The studied single nucleotide polymorphism (SNP) site (A/G) is indicated by an open box. Open arrows represent the location of the forward (for) and reverse (rev) primers in PCR reactions specific for the methylated (M) and unmethylated (U) alleles, respectively. Sequences of these MSP primers are shown (SEQ ID NOS:7-10). Sequence differences between bisulfite-treated DNA and untreated DNA are highlighted in bold italics and sequence differences between methylated (paternally-inherited) and unmethylated (maternally-inherited) DNA are underlined in bold.

Paragraph beginning at line 22 of page 15 has been amended as follows:

MSP assays were modified from the protocol as described by Herman *et al, supra*. The primers M-for (5'-GCGAGCGTAGTATTTTTCGGC-3'; SEQ ID NO:1) and M-rev (5'-AACCAAATAACCTATAAAACCTCTACG-3'; SEQ ID NO:2) were designed for the methylated sequence, while the primers U-for (5'-GTTGTGAGTGTAGTATTTTGGT-3'; SEQ ID NO:3) and U-rev (5'-CAAATAACCTATAAAACCTCTACA-3'; SEQ ID NO:4) were designed for the unmethylated sequence. Five µl bisulfite-treated DNA was added to a 50 µl PCR reaction containing 5 µl 10x TaqMan buffer A (PE Applied Biosystems), 2 mM MgCl<sub>2</sub>, 10 pmol

dNTPs, 20 pmol each of the corresponding MSP primers and 1.25 U AmpliTaq Gold DNA polymerase (PE Applied Biosystems). Reaction mixtures were thermal cycled (methylated allele: 95 °C for 45 sec, 58 °C for 30 sec, 72 °C for 20 sec; unmethylated allele: 95°C for 45 sec, 50 °C for 30 sec, 72 °C for 20 sec) for 45 cycles, with an initial denaturing step of 8 min at 95 °C. PCR products were then analyzed by agarose gel electrophoresis.

Paragraph beginning at line 32 of page 17 has been amended as follows:

The DMR in the human IGF2-H19 locus contains two 450-bp repeat and seven 400-bp repeat units (Nakagawa et al., *Proc Natl Acad Sci U S A* 98:591-596 (2001)) (Fig. 2). An A/G SNP within the DMR (Nakagawa et al., *supra*) was selected as a marker in our investigation (Fig. 2). Polymerase chain reaction (PCR) was used to amplify the SNP in both maternal and fetal DNA samples. Primers were designed using the sequence of the Homo sapiens *H19* gene (Genbank accession number AF125183). Typically, 2 to 5 µl eluted DNA, purified from maternal buffy coat, cord buffy coat or amniotic fluid was added to a 25 µl PCR reaction containing 2.5 µl 10x TaqMan buffer A (PE Applied Biosystems), 3 mM MgCl<sub>2</sub>, 6.26 pmol dNTPs, 5 pmol primers (forward: 5'-ggACGGAATTGGTTGTAGTT-3' (SEQ ID NO:5); reverse: 5'-AGGCAATTGTCAGTTCAGTAA-3' (SEQ ID NO:6)) and 0.625 U AmpliTaq Gold DNA polymerase (PE Applied Biosystems) (95 °C for 8 min followed by 35 cycles of -95 °C -for 1 min, 56 °C for 20 sec, 72 °C for 20 sec). For the forward primer, the nucleotides in upper case corresponded to positions 7927 to 7944 of the *H19* sequence (Genbank accession number AF125183). For the reverse primer, the nucleotides were complementary to positions 8309 to 8329 of the *H19* sequence. PCR products were then analysed by agarose gel electrophoresis and DNA sequencing.

Paragraph beginning at line 26 of page 18 has been amended as follows:

MSP assays were modified from the protocol as described (Herman *et al.* 1996). Five  $\mu$ l bisulfite-treated DNA was added to a 50  $\mu$ l PCR reaction containing 5  $\mu$ l 10X TaqMan buffer A (PE Applied Biosystems), 2.5 mM  $MgCl_2$ , 10 pmol dNTPs, 20 pmol each of the corresponding MSP primers (Fig. 2) and 1.25 U AmpliTaq Gold DNA polymerase (PE Applied Biosystems). The primers M-for and M-rev (Fig. 2; SEQ ID NOS:7 and 8) were designed for the methylated sequence, while the primers U-for and U-rev (Fig. 2; SEQ ID NOS:9 and 10) were designed for the unmethylated sequence. Reaction mixtures were thermal cycled (methylated allele: 95 °C for 45 sec, 55 °C for 20 sec, 72 °C for 20 sec; unmethylated allele: 95°C for 45 sec, 49 °C for 20 sec, 72 °C for 20 sec) for 50 (buffy coat and amniotic fluid DNA) or 56 (plasma DNA) cycles, with an initial denaturing step of 8 min at 95 °C. PCR products were then analyzed by agarose gel electrophoresis. Reaction products were purified using Microspin S-300 HR columns (Amersham Pharmacia) for DNA sequencing or the primer extension assay.

Paragraph beginning at line 11 of page 19 has been amended as follows:

Two  $\mu$ l of the purified MSP product was added to a 25  $\mu$ l reaction containing 50  $\mu$ M ddATP (2',3'-dideoxyadenine triphosphate), 50  $\mu$ M dGTP, 50  $\mu$ M dTTP, 0.2 pmol Cys-5-labeled primer (5'-GGGTTATTTGGGAATAGGATATTTA-3'; SEQ ID NO:11), 4 U Thermo Sequenase (Amersham Pharmacia) and 1.43  $\mu$ l concentrated buffer. Reactions were thermal cycled for 40 cycles (95°C for 30 sec, 51°C for 20 sec, 72°C for 20 sec). The Cys-5-labeled primer was 25 nucleotides (nt) in length and the polymorphic site was 2 nt away from the 3'-end of the primer. For the A allele, the incorporation of the ddATP at this polymorphic site would produce chain termination, thus resulting in an extension product of 27 nt (i.e., 25+2 nt). For the G allele, chain extension would continue until the next A residue which was 5 nt away from the 3'-end of the primer, thus resulting in an extension product of 30 nt (i.e., 25+5 nt). Reaction

products were electrophoresed using a 14% denaturing polyacrylamide gel and analysed using an ALF Express Sequencer (Amersham Pharmacia). Data were analysed by the AlleleLinks program (Amersham Pharmacia).

